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Bending stiffness of lipid bilayers. II. Spontaneous curvature of the monolayers

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Abstract. — In the classical formulation of the elastic bending energy stored in a bilayer, the spontaneous curvatures of the monolayers enter via their sum. Accounting for the spontaneous curvatures of the monolayers individually leads to an essentially different formulation which allows us to explain the following experimental results (i) the decrease in « apparent » bending stiffness of pure bilayers with increasing unsaturation of their lipids and (ii) the instability and hysteresis concomitant with a budding of vesicles induced by a change in temperature. The new formulation predicts above a critical unsaturation of their lipids (i) a micro roughness of the surface and (ii) a spontaneous budding of vesicles in pure bilayers.

1. Introduction.

Natural membranes are composed of many different kinds of molecules and complex models are required to understand their mechanical behaviour. Such models use basic information obtained from pure systems, e.g. lipid-bilayer vesicles of cellular dimensions. But even in these simple systems many observations have not yet been explained. In this paper, we present a new formulation of the elastic bending energy density stored in a bilayer which allows us to understand as yet unexplained experimental results.

The resistance of a bilayer to bending can be decomposed into two contributions which we call bilayer couple bending and single layer bending [1]. Bilayer couple bending arises from the resistance of the two monolayers to a change in surface area and from their fixed interlayer distance. The single layer bending stiffness which is also called the intrinsic bending stiffness arises from the resistance of the molecules making up a monolayer to a change in shape, e.g. from cylindrical to conical. In bilayer couple bending, either the local or the global nature or a mixture of both occurs, depending on the time scale of the deformation [1]. In bilayers composed of a single lipid species single layer bending is local. If more than one lipid species is present single layer bending can also become global.

Idealized shapes have been assigned to lipids to describe their packing behaviour [2]. Phosphatidylcholines (PC) with two saturated hydrocarbon chains are assumed to be almost cylindrical. However, the molecule becomes increasingly inverted cone shaped with increasing degree of unsaturation [3], i.e. the cross sectional area at the headgroup position is
smaller than that at the loose end of the hydrocarbon chains. This can also be achieved by a
decrease in headgroup size. LysoPC which has a single hydrocarbon chain is taken to be cone
shaped, because the cross sectional area of the headgroup is larger than that of the
hydrocarbon chain. The greater the cone angle of such idealized shapes the greater is the
spontaneous curvature of a monolayer composed of corresponding lipids.

Mechanical experiments with lipid vesicles use exclusively symmetric bilayers. The
spontaneous curvature (in single layer bending) of such bilayers vanishes due to the opposite
orientation of the monolayers within the bilayer. For this reason the spontaneous curvature of
the individual monolayers has been neglected in the theoretical treatment of bilayer elasticity.
In this paper the elastic energy density in single layer bending of a bilayer is formulated in
such a way that it accounts for each monolayer separately. In this case two effects are found.
The first is independent of the spontaneous curvature of the monolayers and becomes
relevant at radii of curvature comparable to the thickness of the bilayer. The second is
independent of the radius of curvature and becomes relevant when the radius corresponding
to the spontaneous curvature of the monolayer is comparable to the thickness of the bilayer.

2. Analysis.

The classical theory of thin shells is based on one neutral surface, which is defined as a
conceptual surface within the bilayer the area of which does not change when the bilayer is
bent. Classically the contribution (e) of local single layer bending to the elastic energy density
(energy per surface area) of a lipid bilayer is:

\[ e = \frac{B_s}{2} \left( \frac{1}{r_1} + \frac{1}{r_2} - 2 \xi_s \right)^2 \]  

(1)

Here \( r_1 \) and \( r_2 \) are the principal radii of curvature. The bending energy originating from
bilayer couple bending is not included in equation (1), as \( B_s \) is the intrinsic (or single layer)
bending stiffness of the bilayer, and \( \xi_s \) its spontaneous curvature in single layer bending. The
definition of \( \xi_s \) differs by a factor of 2 from the one suggested by Helfrich [4], for consistency
[1].

We now decompose the bilayer in its two monolayers. They are characterized by indices o
or i, representative for the outside or inside of a closed vesicle, respectively. Each monolayer
is assigned its own neutral surface. It is defined as above whereby we assume we can bend
each monolayer separately. The isotropic tensions arising in each monolayer from bilayer
couple bending are assumed to be linearly superposable to the stresses arising from single
layer bending. The distance of the two neutral surfaces from the midsurface of the bilayer are
\( \delta_o \) and \( - \delta_i \), respectively. If the bilayer is bent the radii of curvature of the two monolayers
have different values. To distinguish quantities in the new analysis (which accounts for the
monolayers individually) from quantities in the classical analysis (Eq. (1)), we add a tilde to
the symbols. For the energy density of the outer layer (\( \tilde{\xi}_o \)) we obtain:

\[ \tilde{\xi}_o = \frac{\tilde{B}_o}{2} \left( \frac{1}{r_1 + \delta_o} + \frac{1}{r_2 + \delta_o} - 2 \tilde{\xi}_o \right)^2, \]  

(2)

where \( \tilde{B}_o \) and \( \tilde{\xi}_o \) are the bending stiffness and spontaneous curvature of the outer layer,
respectively. The index s is dropped for simplicity. It is obvious that these are quantities in
single layer bending. For the inner layer the same formula applies, with the index o replaced
by i. Here the sign convention is the same for \( \tilde{\xi}_o \) and \( \tilde{\xi}_i \) irrespective of the orientation of the
monolayers within the bilayer. From the balance of bending moments we obtain the spontaneous curvature of the bilayer as a weighted sum:

\[ \xi_s = \frac{\tilde{B}_o \tilde{\xi}_o + \tilde{B}_1 \tilde{\xi}_1}{\tilde{B}_o + \tilde{B}_1} \]  

(3)

This is analogous to the unstretched length of a parallel arrangement of springs. The energy density of the bilayer is given by the sum:

\[ \tilde{e} = \tilde{e}_o + \tilde{e}_1. \]  

(4)

To simplify the expressions we set \( \delta_o = -\delta_1 = \delta \) and \( \tilde{B}_o = \tilde{B}_1 = \tilde{B}/2 \). First we treat the case \( \tilde{\xi}_o = \tilde{\xi}_1 = 0 \). The elastic energies calculated under the last assumption are characterized by the index \( v \). After expanding in a Taylor series and neglecting terms with powers larger than 2 for the ratio \( \delta/r \) we obtain after some algebra:

\[ \tilde{e}_v = \frac{\tilde{B}_s}{2} \left\{ \left( \frac{1}{r_1} + \frac{1}{r_2} \right)^2 + 3 \delta^2 \left( \frac{1}{r_1^4} + \frac{1}{r_2^4} \right) + 2 \delta^2 \left( \frac{1}{r_1^2 r_2} + \frac{1}{r_1 r_2^2} \right) \right\}. \]  

(5)

The last two terms contain corrections which are positive and quadratic in \( \delta/r \). Consequently they become significant when the radii of curvature are comparable to the thickness of the bilayer.

We now treat the case of non zero spontaneous curvature of the monolayers. After some more algebra we obtain:

\[ \tilde{e} = \tilde{e}_v + \tilde{B}_s \left\{ \tilde{\xi}_o^2 + \tilde{\xi}_1^2 - (\tilde{\xi}_o + \tilde{\xi}_1) \left( \frac{1}{r_1} + \frac{1}{r_2} \right) + \delta (\tilde{\xi}_o - \tilde{\xi}_1) \left( \frac{1}{r_1^2} + \frac{1}{r_2^2} \right) - \delta^2 (\tilde{\xi}_o + \tilde{\xi}_1) \left( \frac{1}{r_1^3} + \frac{1}{r_2^3} \right) \right\}. \]  

(6)

We consider the special case : \( \tilde{\xi}_o = \tilde{\xi}_1 = \tilde{\xi}_q \) and call the corresponding energy density \( \tilde{e}_q \). Equation (6) reduces to:

\[ \tilde{e}_q = \tilde{e}_v + 2 \tilde{B}_s \left\{ \tilde{\xi}_q^2 - \tilde{\xi}_q \left( \frac{1}{r_1} + \frac{1}{r_2} \right) - \delta^2 \tilde{\xi}_q \left( \frac{1}{r_1^2} + \frac{1}{r_2^2} \right) \right\}. \]  

(7)

We have three terms in addition to equation (5). The first two are expected from equation (1) since according to equation (3) : \( \xi_s = \tilde{\xi}_q \). The third is quadratic in \( \delta/r \). This means that, as for equation (5), \( \tilde{\xi}_q \) is different from \( \tilde{e} \) only when the radii of curvature become comparable to the thickness of the bilayer.

The case of interest is the symmetric bilayer, where the spontaneous curvatures of the two monolayers have opposite values. We set:

\[ \tilde{\xi}_o = -\tilde{\xi}_1 = \tilde{\xi}_p. \]  

(8)

From the sign convention implicit in equation (2) and (8) it can be appreciated that inverted cone shaped lipids have negative \( \tilde{\xi}_p \). A cylindrical shape corresponds to \( \tilde{\xi}_p = 0 \). Conical lipids
are characterized by \( \tilde{\xi}_p = 0 \). The energy density for the symmetric bilayer is called \( \tilde{\varepsilon}_p \). Equation (6) reduces to:

\[
\tilde{\varepsilon}_p = \tilde{\varepsilon}_v + 2 \tilde{B}_s \left\{ \tilde{\varepsilon}_r^2 + \tilde{\xi}_p \delta \left( \frac{1}{r_1^3} + \frac{1}{r_2^3} \right) \right\}.
\] (9)

We have two terms containing \( \tilde{\xi}_p \). The first is independent of \( \delta \) and \( r \). If \( \tilde{\xi}_p \) is uniform on the surface the integration of this term over the surface gives a constant value independent of the shape of the vesicle.

The second term is a correction term that is linear in \( \delta \). The size of the correction is independent of the radii of curvature. It becomes relevant when \( |\tilde{\xi}_p \delta| \) cannot be neglected as compared to unity. The energy density decreases for \( \tilde{\xi}_p < 0 \) and increases for \( \tilde{\xi}_p > 0 \).

We consider two special cases of equation (9). First, the deformation into a spherical cap \( (r_1 = r_2 = r_c) \). We call the respective energy density \( \tilde{\varepsilon}_c \) and we obtain:

\[
\tilde{\varepsilon}_c = 2 \tilde{B}_s \left\{ \frac{1}{r_c^2} + \frac{3}{r_c^4} \tilde{\xi}_c^2 + 2 \tilde{\xi}_p \frac{\delta}{r_c} \right\}.
\] (10)

In contrast to equation (1) there is no value of \( r_c \) for which \( \tilde{\varepsilon}_c \) becomes zero. If we now consider the deformation in a saddle with \( r_1 = -r_2 = r_d \) and call the respective energy density \( \tilde{\varepsilon}_d \), we obtain:

\[
\tilde{\varepsilon}_d = 2 \tilde{B}_s \left( \frac{\delta}{r_d^2} + \tilde{\xi}_p \right)^2.
\] (11)

In equation (11) the energy density vanishes for negative \( \tilde{\xi}_p \) when \( r_d^2 = -\delta/\tilde{\xi}_p \).

3. Discussion.

Two kinds of modification of the classical energy density (Eq. (1)) have been found by accounting for the monolayers individually. The first is a correction consisting of several terms which become relevant when \((\delta/r)^2\) becomes comparable to 1.

Equation (1) assigns zero elastic energy to a state of internal stress which prevails if \( \tilde{\xi}_o \neq \tilde{\xi}_i \). This is corrected by the second modification. It consists of two terms which can be intuitively understood as follows. Figure 1B shows schematically symmetric bilayers in cross section. No external bending moments are acting and hence they are flat. Figure 1A shows two cases for the spontaneous curvature of the monolayers, corresponding to cone (\( \beta \)) and inverted cone (\( \alpha \)) shaped lipids. It is obvious that the energy stored in the flat configuration is the same in both cases, provided \( \tilde{B}_s \) is identical. This energy corresponds to the term in equation (9) which is quadratic in \( \tilde{\xi}_p \).

In figure 1C both bilayers are bent by external moments into a spherical cap with a radius larger than that corresponding to the spontaneous curvature. For case \( \alpha \) this means the energy stored in the upper layer increases and in the lower one it decreases. If we neglect the difference in curvature between the two layers we obtain a net increase in energy which is proportional to the square of the curvature imposed in figure 1C. This increase can be calculated from equation (1) with \( \tilde{\xi}_s = 0 \). Taking into account the difference in curvature
between the two layers makes the energy increase in the upper layer smaller and the decrease in the lower layer larger. This leads to a reduction in energy which corresponds to the term in equation (9) which is linear in \( \tilde{\varepsilon}_p \). By an analogous argument we find that the energy increases in case \( \beta \).

In modelling equilibrium shapes by minimization of the elastic energy the first term only becomes relevant when \( \tilde{\varepsilon}_p \) is not uniform on the surface. This can occur by lateral phase separation provided lipid species of different intrinsic shapes are present.

The second term is relevant in pure as well as in mixed bilayers. It is the larger the greater the difference in the spontaneous curvature of the two monolayers. For symmetric bilayers it becomes relevant when \( \left| \tilde{\varepsilon}_p \delta \right| \) cannot be neglected as compared to unity. Recent measurement of the geometry of lipid structures in excess hydrophilic and excess hydrophobic solvent allowed for the first time a measurement of \( \tilde{\varepsilon}_p \). Two values have been published: \(-1/(3.15 \text{ nm})\) for a DOPE monolayer and \(-1/(3.78 \text{ nm})\) for a 3/1 mixture of DOPE and DOPC [5]. If we use 1 nm (one quarter of the bilayer thickness) for \( \delta \) we obtain \( \left| \tilde{\varepsilon}_p \delta \right| \approx 0.3 \). This shows that there is an appreciable correction to the energy density in bending for frequently used bilayers.

The experimental value of \( \tilde{\varepsilon}_p \) used above was adopted from the geometry of inverted hexagonal phases in water and tetradeacne as solvents [5]. In a bilayer there is no organic solvent. It can therefore be argued that the absolute value of \( \tilde{\varepsilon}_p \) in a bilayer and consequently its influence is much smaller than envisaged above. There comes, however, circumstantial evidence from the measurements on the bending stiffness of lipid bilayers. In the first paper of this series [1] it was noted that the ratio between the isotropic modulus \( (K) \) and the bending stiffness in single layer bending \( (\mathcal{B}_1) \) was not independent of the type of lipid used.

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**Fig. 1.** — Schematical drawing of the spontaneous curvatures of two types \( (\alpha : \tilde{\varepsilon}_p \approx 0, \beta : \tilde{\varepsilon}_p > 0) \) of monolayers (A), the (symmetric) bilayer formed from these monolayers without external bending moments present (B), the bilayer bent by external bending moments (C). For details see text.
\(B_s/K\) had roughly half the value for lipids containing 6 or 8 double bonds than lipids containing just one or none \([6]\). According to a continuum mechanical model a constant ratio was expected \([1]\). If we used equation (9) to fit the experimental results instead of equation (1) we could make the ratio \(\tilde{B}_s/K\) constant by choosing appropriate values for \(\tilde{\xi}_p\) (\(\tilde{\xi}_p \approx 0\) for the lipids with saturated and \(\tilde{\xi}_p \delta \approx -0.5\) for the lipids with the multiunsaturated hydrocarbon chains). This choice is in qualitative agreement to the trend of \(\tilde{\xi}_p\) with the degree of unsaturation.

The introduction of \(\tilde{\xi}_p\) seems to increase the number of elastic constants, but we show that this is not the case. In the classical description 3 independent parameters are necessary to describe the elastic behaviour of symmetric bilayers. One possible choice is \(K, B_v,\) and \(B_c\) the bending stiffness in bilayer couple bending. Another choice is \(K, B_v,\) and \(\delta\) since \(B_c\) can be expressed by \(K\) and \(\delta\) \([1]\). In the new description the parameters would be \(K, \delta,\) and \(\tilde{\xi}_p\) since \(\tilde{B}_s\) is chosen to depend on \(K\). As a further refinement \(\tilde{B}_s\) could depend on \(\delta\) as well.

For the special case that the time scale and the geometry of the deformation are such that bilayer couple bending does not contribute \([1]\), \(B_s\) as obtained from fitting to equation (1) is useful as an apparent bending stiffness that describes the observation that bilayers of lipids with unsaturated hydrocarbon chains bend more easily than those with saturated ones. But even in this special case the use of equation (1) has two drawbacks. First, the value of \(B_s\) depends on the geometry of the bilayer since the curvatures enter differently in equation (1) and (9) (even if we neglect the terms proportional to \(\delta^2\)). Second, at curvatures comparable in absolute value to \(|\tilde{\xi}_p|\) new effects emerge that remain hidden when only equation (1) is used. Some of these effects are described in the next chapter.

4. Applications.

4.1 Micro roughness. — To explain their results in bilayer adhesion Helfrich and coworkers \([7, 8]\) postulated a corrugated bilayer surface. To support this hypothesis Helfrich proposed that this was due to energy terms proportional to the 4th power in curvature \([9]\). The theory developed in this paper suggests that terms proportional to the second power of the curvature are sufficient.

As a simple model for a corrugated surface we consider a square 2D lattice of alternating elevations and depressions. The lattice constant is assumed to be small compared with the radius of the vesicle. This means aside from the corrugations the bilayer can be considered to be flat. In the center of each square is a saddle point. On the corners are the elevations and the depressions. With respect to the energy density, the elevations and depressions are equivalent. This means for each saddle point there is one elevation either oriented outward or inward.

To make a rough estimate of whether a micro roughness can exist in static equilibrium, we calculate whether, when starting from the flat configuration bending energy is released in a saddle deformation that is now available for the formation of a spherical cap. To this purpose we add up the two contributions given by equations (10) and (11) and call it \(\tilde{\xi}_{c+d}\). A negative value of \(\tilde{\xi}_{c+d}\) would indicate a stable corrugated configuration. Setting \(r_c = r_d = r\) we get:

\[
\tilde{\xi}_{c+d} = \frac{2 \tilde{B}_s}{r^2} \left[ 1 + 4 \frac{\delta^2}{r^2} + 4 \tilde{\xi}_p \delta \right].
\]

We treat the case of pure bilayers. \(\tilde{\xi}_p\) can therefore considered to be uniform on the surface. Consequently the terms proportional to \(\tilde{\xi}_p^2\) are omitted in equation (12). Bilayer couple
bending is not expected to play an appreciable role since the mean curvature averaged over the bilayer surface does not change when the corrugations form.

We now look for radii \( r_m \) that make \( \varepsilon_{c+d} \) minimal. The derivative of \( \varepsilon_{c+d} \) with respect to \( r \) vanishes at \( r_m^2 = -8 \xi_p (1 + 4 \xi_p \delta) \). For \( \xi_p \delta \ll 1/4 \) this equation has real solutions and the second derivative becomes positive, indicating a minimum. Assuming \( \delta = 1 \text{ nm} \) we obtain \(-1/(4 \text{ nm})\) for the critical value for \( \xi_p \) below which a minimum exists. Figure 2 shows the dependence of \( r_m \) as a function of \( \xi_p \). Naturally these values do not give the actual radii of a corrugated surface. In a rigorous calculation the minimization must be done for the whole surface. In addition the bending energy postulated to be associated with a saddle deformation [10] would have to be included. Therefore \( r_m \) and the critical value of \( \xi_p \) may have quite different values in real bilayers.

![Figure 2](image_url)

Fig. 2. — Radius \( r_m \) and energy density \( \varepsilon_{c+d} \) of a corrugated (symmetric) bilayer as a function of \( \xi_p \), the spontaneous curvature of the monolayers. For details see text.

According to chapter 3, \( B_s \) for lipids with unsaturated hydrocarbon chains corresponds to \( B_s \) for lipids with saturated hydrocarbon chains. To calculate \( \varepsilon_{c+d}(r_m) \) from equation (12) we use \( 1.15 \times 10^{-12} \text{ erg} \), the value for \( B_s \) of DMPC bilayers [11]. The values are plotted in figure 2. The energy density of a corrugated bilayer may be an order of magnitude smaller since the saddles and elevations are expected to contribute much more than the regions inbetween. Nevertheless we can see from the trend that the energy density stabilizing the corrugation strongly increases with decreasing \( \xi_p \). Adhesion of flaccid bilayers to locally flat substrates requires adhesive energy densities larger than these values.

A flattening of postulated corrugations by isotropic tensions was suggested by Helfrich and coworkers [7, 8] to be responsible for their observation of tension dependent adhesion. The data in figure 2 suggest that below a certain value of \( \xi_p \) the energy stabilizing the corrugations is as large as predicted by these authors. However, if two bilayers composed of the same lipids are under the same isotropic tension the corrugations in both bilayers should be identical. This should allow a close apposition and therefore a tension independent adhesion.
4.2 Vesiculation. — Vesiculation implies the budding of daughter vesicles from a mother vesicle. Two kinds of vesiculation have been observed. The first occurs spontaneously provided there is excess surface area [6]. In the second the mother vesicle is stable despite an excess surface area but budding can be induced by an increase in temperature [12]. Both mechanisms can be explained based on the concepts developed above.

4.2.1 Spontaneous vesiculation. — The mechanism is demonstrated by means of an example. We begin with a spherical mother and reduce the volume slightly by a mechanism that is not important for the argumentation. We assume that a small daughter vesicle (with radius \( R_d \)) buds from the mother which is then spherical (with radius \( R_m \)). The budding process occurs spontaneously when the bending energy is lower for the budded state. Two contributions to the energy are considered: the first arising from single layer bending (Eq. (9)) and the second from bilayer coupling bending [1]. In the third paper of this series [10] an additional elastic contribution is postulated which is due to a deformation of lipid molecules which does not preserve their idealized axisymmetric shape. This contribution is not taken into account as it does not change the qualitative conclusion of the analysis presented below.

The energy stored in single layer bending is obtained by integration of the energy density over the surface. For the spherical portions, we use equation (10). We assume the diameter of the daughter to be large enough to neglect the term with \( \delta^2 \). The \( \tilde{\delta}_p^2 \) term is omitted since we treat pure bilayers. Small impurities would not change the result. The contribution of each sphere (\( \tilde{E}_s \)) is the same

\[
\tilde{E}_s = 8 \pi \tilde{B}_s (1 + 2 \tilde{\delta}_p \delta).
\]

As to the neck, we assume that this region has the shape of the inner half of a torus. The clear span \( (N) \) of the torus and its thickness parallel to its axis of symmetry are assumed to be equal. For simplicity, we assume the curvature to have everywhere the same value as on the midline of the torus. We then obtain an expression for the energy stored in the neck \( (\tilde{E}_n) \) from equation (11)

\[
\tilde{E}_n = 4 \pi \tilde{B}_s (\pi - 2) \left( \frac{\delta}{N} \right)^2 + 2 \tilde{\delta}_p \delta.
\]

For simplicity we assume the elastic energy stored in the mother before budding to be the same as if the mother was spherical although this would not allow budding to take place due to the constant volume restriction. The energy difference due to single layer bending before and after budding \( (\Delta \tilde{E}_s) \) is then the sum of two contributions

\[
\Delta \tilde{E}_s = \tilde{E}_s + \tilde{E}_n.
\]

As to the energy due to bilayer coupling bending, we assume it to be zero before the volume was reduced. This is reasonable if the mother vesicle was grown at the same temperature at which the experiment was performed. If it was not the case the final conclusion would not change. The assumption defines the average spontaneous curvature in bilayer coupling bending to be \( (R_m^2 + R_d^2)^{-1/2} \). In the budded state the local values of the spontaneous curvature in bilayer coupling bending are different from the average value. Taking advantage from the fact that in a sphere the energy density in local and global bending is the same we obtain:

\[
\tilde{\xi}_m - \tilde{\xi}_d = - \frac{R_m - R_d}{R_m R_d},
\]
where $\xi_m$ and $\xi_d$ denote the spontaneous curvatures in bilayer couple bending in the mother and daughter, respectively. For the increase in elastic energy due to bilayer couple bending ($\Delta E_c$) concomittant with budding we obtain:

$$\Delta E_c = 8 \pi B_c \left\{ (1 - R_m \xi_m)^2 + (1 - R_d \xi_d)^2 \right\}. \tag{17}$$

Here $B_c$ is the bending stiffness in bilayer couple bending.

Spontaneous budding is expected when the sum $\Delta E_s + \Delta E_c$ becomes negative. To make a rough estimate, we take 1 nm for $\delta$ and arbitrarily 10 nm for $N$ and as representative values $R_m = 10$ $\mu$m and $R_d = 0.2$ $\mu$m. From the average spontaneous curvature and equation (16) we obtain $\xi_m$ and $\xi_d$. For $B_c$ we take $3 \bar{B}_s$ [1]. Then $\Delta E_s + \Delta E_c$ becomes negative when $\bar{\xi}_p < -1/(3.1 \text{ nm})$.

The assumptions made on the geometry of the neck region and the diameter of the daughter vesicle were arbitrary. Using the correct values might give a different number for the critical value of $\bar{\xi}_p$ below which spontaneous vesiculation is expected. Unfortunately the kinds of lipid for which it was observed were not specified [6].

It can be shown that $\Delta E_c$ decreases when we decrease $R_d$ while keeping the total surface area constant. This explains why very small vesicles are observed [6]. To determine the equilibrium value of $R_d$ the $\delta^2$ term from equation (10) would have to be carried over to equation (13).

It is observed [6] that after budding the daughters remain connected to the mother. Complete separation would require an energy input of $-\tilde{E}_n$. For a numerical estimate we insert the numbers for $\delta$ and $N$ as above. For $\bar{B}_s$ we use $28 kT$ [11] and for $\bar{\xi}_p$ the critical value $-1/(3.1 \text{ nm})$. We obtain $-\tilde{E}_n = 260 kT$. This is much larger than thermal energies and explains the observations.

4.2.2 Vesiculation upon temperature change. — If $\bar{\xi}_p$ is larger than the critical value no spontaneous vesiculation is expected. However, vesicles made of lipids with saturated hydrocarbon chains ($\bar{\xi}_p = 0$) change their equilibrium shape towards an exo- or endovesiculated state when the temperature is increased [12]. To explain this finding bilayer couple bending was invoked and sequences of shapes were successfully modelled [13]. The budding itself is often accompanied by an instability which could not be modelled with this analysis. Upon recouling a hysteresis was observed in that a non budded equilibrium shape was attained at a temperature ($T_e$) appreciably below the one where budding occurred during warming.

We propose impurities with one or several double bonds per hydrocarbon chain to be responsible for the instability and hysteresis. At a critical value of internal stress we suggest that these molecules accumulate in the neck region in a process characterized by positive feedback. The decrease in total free energy would be equal in absolute value to the increase in elastic energy during warming from $T_e$ to the temperature where budding occurred. To calculate this energy difference would require a fitting procedure which is beyond the scope of this paper.

The transitory opening of the neck upon recouling has been explained [12] by the decrease in surface area of the vesicle and the constancy in its volume. The reformation of the neck can be explained by a much larger local concentration of the impurities than in the warming process.
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